Amendments to the CLAIMS

This listing of claims will replace all prior versions, and listing, of claims in the application.

Listing of Claims

1 – 5 (canceled).

6. (currently amended). A method for determining the presence and/or quantity of a modified or unmodified target polypeptide in at least one mixture of different polypeptides, comprising:

a) providing a mixture of different polypeptides;
b) adding a known quantity of a single peptide internal standard labeled with a mass-altering label, thereby generating a spiked mixture, wherein the labeled peptide internal standard comprises a subsequence of the target polypeptide and wherein the labeled peptide internal standard possesses a known peptide fragment signature diagnostic of the presence of the peptide;
c) treating the spiked mixture with a protease activity to generate a plurality of peptides including the labeled peptide internal standard and peptides corresponding to the target polypeptide;
d) fragmenting the labeled peptide internal standard and any target peptide present in the spiked mixture comprising the same amino acid sequence as the labeled peptide internal standard;
e) determining the ratio of labeled fragments to unlabeled fragments; and
f) calculating from the ratio and the known quantity of the labeled internal standard, the quantity of the target polypeptide in the mixture.

7. (original). The method of claim 6, wherein the fragmenting is performed by multistage mass spectrometry.

8. (original). The method of claim 6, further comprising separating peptides obtained in step (c) using a chromatography step.

9. (original). The method according to claim 8, wherein the chromatography step comprises performing HPLC.

10. (original). The method according to claim 9, wherein the labeled peptide internal standard and target peptide comprising the same amino acid sequences as the labeled peptide internal standard are co-eluted during separation.

11. (original). The method according to claim 6, wherein the mixture of different polypeptides is selected from the group consisting of: a crude fermenter solution, a cell-free culture fluid, a cell or tissue extract, blood sample, a plasma sample, a lymph sample, a cell or tissue lysate; a mixture comprising at least about 100 different polypeptides; a mixture comprising substantially the entire complement of proteins in a cell or tissue.

12. (original). The method according to claim 6, wherein the peptide internal standard is labeled using a stable isotope.

13. (previously presented). The method according to claim 6, wherein the labeled peptide internal standard is produced according to a method for generating a peptide internal standard, comprising:

a) identifying a real or predicted peptide digestion product of a target polypeptide;
b) determining the amino acid sequence of the peptide;
c) synthesizing a peptide comprising the amino acid sequence of the peptide digestion product;
d) labeling the peptide with a mass-altering label;
e) fragmenting the peptide and identifying a peptide signature diagnostic of the peptide.

14. (original). The method according to claim 6, wherein the presence and/or quantity of target polypeptide is diagnostic of a cell state.

15. (original). The method according to claim 14, wherein the cell state is representative of an abnormal physiological response.

16. (original). The method according to claim 15, wherein the abnormal physiological response is diagnostic of a disease.

17. (original). The method according to claim 14, wherein the cell state is a state of differentiation.

18. (original). The method according to claim 6, further comprising determining the presence and/or quantity of target peptides in at least two mixtures.

19. (original). The method according to claim 18, wherein one mixture is from a cell having a first cell state and the second mixture is from a cell having a second cell state.

20. (original). The method according to claim 20, wherein the first cell is a normal cell and the second cell is from a patient with a disease.

21. (original). The method according to claim 18, wherein the determining is done in parallel.

22. (original). The method according to claim 18, wherein the two mixtures are the same and the labeled peptide internal standard is provided in different known amounts in each mixture.

23 – 24 (canceled).

25. (original). The method according to claim 18, wherein the labeled peptide internal standard in each mixture comprises the same peptide but different labels.

26 - 46 (canceled).